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Risk Assessment of Complex Mixtures

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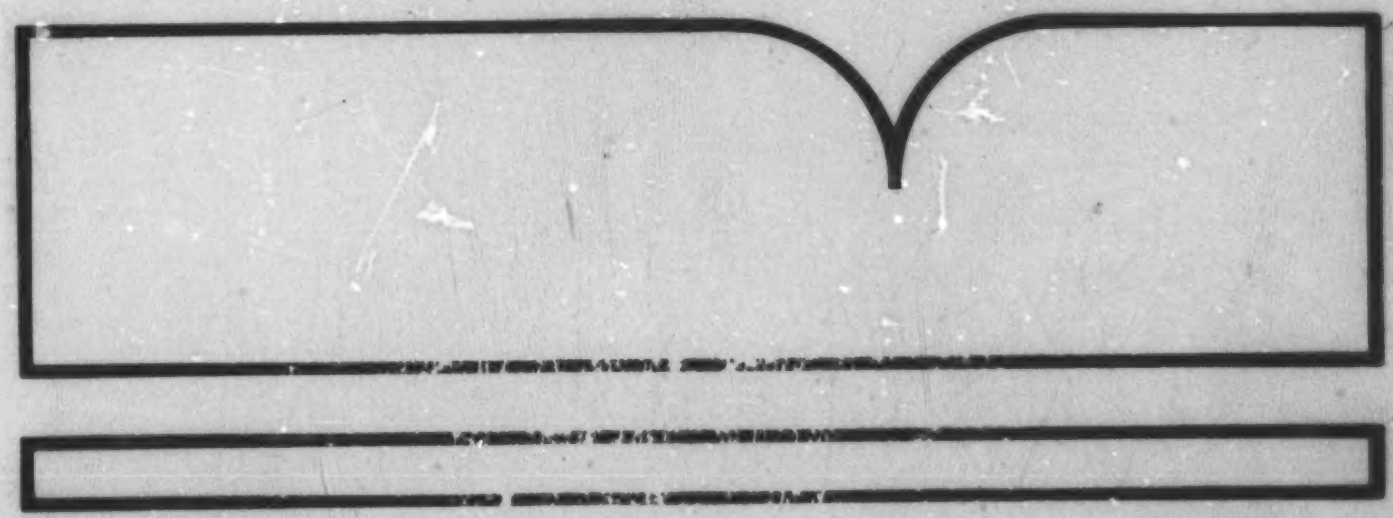

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RISK ASSESSMENT OF COMPLEX MIXTURES

by

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16. ABSTRACT

Quantitative carcinogenic assessment of complex mixtures is complicated by the variability of the mixtures. Different samples of effluents from the same source may vary in their carcinogenic potency. A multiplicative effect could be produced by the action of different fractions of a mixture on different stages of the carcinogenic process. A mathematical formula explaining this multiplicative effect is presented here. Antagonism may result from the action of different fractions on the same stage. Actual data from both epidemiologic studies and animal investigations are used for illustration.

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RISK ASSESSMENT OF COMPLEX MIXTURES

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INTRODUCTION

Risk assessment of suspected carcinogens involves both a qualitative and quantitative evaluation. The qualitative evaluation evaluates the relevant animal, epidemiologic, mutagenic, and cell transformation studies as to the likelihood that the agent is a human carcinogen.

The quantitative evaluation is an estimate of the carcinogenic potency of the suspected carcinogen. Potency is derived by fitting a mathematical model to the dose-response data from either animal or epidemiologic studies in an attempt to describe what an estimate of the risk would be at low doses. Short-term genetic bioassay data, which is the focus of this symposium, are not used for carcinogenic risk estimation, although such data have been used in some instances to provide an idea of the comparative carcinogenic potency of different compounds. Several types of risk estimation models have been used for animal dose-response data. Most of the models that have

been used for the epidemiologic data are derivatives of the multi-stage model.

The use of animal data for human risk assessment has several limitations:

1. Animals may respond differently than humans as a result of metabolic or other species-specific differences.
2. The animals are tested at high doses, usually doses that humans would not encounter.
3. The lifetime testing to which animals are subjected is not the equivalent of a human lifetime.

Many limitations are also encountered in the use of epidemiologic data for quantitative evaluation, however. These include:

1. Lack of exposure data for the time period of concern.
2. Small sample sizes and short follow-up periods in the case of cohort studies.
3. Confounding exposures to other carcinogens.

In most cases, however, epidemiologic or animal data on complex mixtures simply does not exist. In lieu of such data, a comparative potency approach in short-term bioassays, as indicated earlier, has been proposed. By this approach, a unit cancer risk estimate, or in other words, the risk at unit dose (e.g., 1 μg/L), is calculated

for a mixture based on its potency in a short-term bioassay relative to that of a mixture for which a unit risk has been calculated. Albert et al. (1983) found that the relative potencies by skin tumor initiation in SENCAR mice of coke oven emission extracts, roofing tar emission extract, and cigarette smoke condensate appeared to correlate well with the relative carcinogenic potencies based on epidemiologic data. The limitation of this bioassay is that potency as determined by a skin tumor initiation bioassay may not correlate well with the potency of the mixture in a cancer bioassay (e.g., the mixture may have both initiation and promotion potential such that the complete carcinogenic potency of the mixture is quite different from its tumor initiation potential). The mathematical implications of the multistage theory with regard to the carcinogenic action of a complex mixture are explored here. Actual data from both epidemiologic studies and animal investigations are used for illustration. Regulatory ramifications of this discussion are also addressed.

RISK ASSESSMENT OF COMPLEX MIXTURES

The main problem associated with doing risk assessments of complex mixtures is that the chemical profiles, and thus the carcinogenic interaction of the mixtures, may vary from source to source. To eventually be able to assess the risk of a population

exposed to a complex mixture with a reasonable amount of confidence, it is necessary that we better understand some of the carcinogenic mechanisms involved. Several studies have examined the synergistic and antagonistic effects of chemicals in a mixture with regard to carcinogenicity. Some examples of these studies, both human and animal, are reported in Tables 1 and 2.

In an attempt to explain these phenomena, we will apply the theory of multistage carcinogenesis to interpret and evaluate the data obtained from the animal and human studies. The multistage theory of carcinogenesis, though oversimplified in our example, does offer considerable plausibility for interpreting the dose-response data obtained from animal experiments and epidemiologic studies. However, it is important to understand the underlying assumptions and limitations. For instance, the multistage model assumes that the transition rate from one stage to the next stage is independent of age. While this assumption has been shown by Peto et al. (1975) to be true for B[a]P-induced skin cancer in animals, it may not be true for other carcinogen-induced cancers. For instance, with regard to human breast cancer data, the transition rates may vary with hormone levels which are closely related to the age of the individuals. Another possibility that is not included in the simple multistage theory, although the model can be extended to accommodate, is that the promotion and/or inhibition activity of

environmental factors other than the factor under study may have an effect on the proliferation rates of partially or completely transformed cells.

The simple multistage model assumes that a cell is capable of generating a malignant neoplasm when it has undergone k changes in a certain order. The rate, r_i, of the i^{th} change is assumed to be linearly related to D(t), the dose at age t, i.e., $r_i = a_i + b_i D(t)$, where a_i is the background rate and b_i is the proportionality constant for the dose (Figure 1). It can be shown (Crump and Howe, 1984) that the probability of cancer by age t is given by

$$P(t) = 1 - \exp\,[-H(t)]$$

where

$$H(t) = \int_0^t \int_0^{u_k} \cdots \int_0^{u_2} \{\,[a_1 + b_1 D(u_1)] \cdots [(a_k + b_k D(u_k)]\,\}\, du_1 \cdots du_k$$

is the cumulative incidence rate by time t.

When H(t) or the risk of cancer is small, P(t) is approximately equal to H(t). When only one stage is dose-related, all proportionality constants are zero except for the proportionality constant for the dose-related stage. The implications of the model when one stage is carcinogen-affected has been summarized by Brown and

Chu (1983) as follows:

> For exposure at a near constant level to a carcinogen, the multistage theory predicts the following patterns of excess risk: (1) For any affected stage, excess risk will increase with increasing level and/or duration of exposure; (2) if only the first stage is affected, for fixed exposure duration, excess risk is independent of age at start of exposure and is an increasing function of time since exposure stopped; and (3) if only the penultimate stage is affected, for fixed exposure duration, excess risk is an increasing function of age at start of exposure and is independent of time since exposure stopped.

Since a complex mixture often contains more than one carcinogen, the likelihood is increased that the mixture will act on more than one stage of the carcinogenic process. Without loss of generality in our discussion, assume that two stages, the m^{th} and n^{th} ($1 \leq m < n \leq k$), are dose-related. Then,

$$H(t) = H_0 + H_1 + H_2 + H_{12}$$

where

$H_0 = (a_1 a_2 \ldots a_k) t^k / k!$

$H_1 = (a_1 a_2 \ldots a_k)(b_m / a_m) \int_0^t \int_0^{u_k} \ldots \int_0^{u_2} D(u_m) du_1 \ldots du_k$

H_2 is similar to H_1 except that subscript m is replaced by n

$H_{12} = (a_1 a_2 \ldots a_k)(b_m b_n / a_m a_n) \int_0^t \int_0^{u_k} \ldots \int_0^{u_2} D(u_m) D(u_n) du_1 \ldots du_k.$

That is, the cumulative incidence function H(t) can be decomposed into four components: H_0 is the background cumulative incidence,

H_1 and H_2 are cumulative incidences when only one stage is dose-related, and H_{12} is the multiplicative term related to the multiple of the two dose-related rates of change. When the two stages are affected separately by two different carcinogens (e.g., B[a]P and a non-B[a]P carcinogen in the mixture) then the multiplicative term reflects the synergism due to the two carcinogens. Obviously, the multiplicative effect would not exist if one of the two compounds were removed. When the same stage is affected by different agents, the synergistic effect does not occur under the simple multistage theory, but antagonism may occur due to the competition of carcinogens for the partially transformed cells of a particular stage. These conclusions may not hold if the transition rate from one stage to the next is modified due to external influences (e.g., breast cancer associated with hormonal change).

The above theoretical discussion suggests that exposure to a complex mixture may produce synergistic and/or antagonistic effects. Thus, the multistage theory can be used to interpret the synergistic and antagonistic observations in humans and animals described earlier.

An illustrative example would be that of Doll and Hill's dose-response data (1956, 1964) for lung cancer and cigarette smoking in British doctors. These data were analyzed by Doll (1971a) and were

presented before the Royal Statistical Society in December 1970. Doll found that the age-specific lung cancer mortality rate for smokers is approximately proportional to the 5^{th} power of duration since the start of exposure and is linearly related to the amount smoked. This implies that smoking affects an early stage of lung carcinogenesis. Following Doll's presentation, Armitage raised the issue of whether smoking affects the early or late stage of carcinogenesis. Armitage stated:

> In this connection, I have always been somewhat puzzled about the effect of cigarette smoke as a carcinogen. The dose-response relationship seems to be linear, which suggests that the carcinogen affects the rate of occurrence of critical events at one stage, and one only, in the induction period. . . . On the other hand, the halt in risk quite soon after smoking stops suggests that a late stage is involved. . . .

The Armitage view can best be seen from Figure 2, which was reported by Doll (1971b) after his presentation of the earlier paper. The fact that the lung cancer rate for the ex-smokers decreased and then increased again approximately 15 years after smoking stopped suggests that an early stage and a late stage are affected by the cigarette smoke. In a subsequent analysis, Doll and Peto (1978) suggested that a quadratic dose-response relationship seems to be preferred to the linear dose-response relationship, suggesting that more than one stage is dose-affected as was observed by Armitage 14 years ago.

Pershagen (1982) recently found that cigarette smoking and exposure to arsenic had a synergistic effect with regard to carcinogenesis. Brown and Chu (1983) concluded from studies of smelter workers that arsenic is a late-stage carcinogen in the multistage model. Brown and Chu found that excess lung cancer risk among smelter workers was an increasing function of age at the start of exposure, and for individuals greater than or equal to 55 years of age, the risk was independent of the time since exposure stopped. This follows the pattern for a late-stage carcinogen as discussed earlier. It is theorized that older individuals are at a greater risk of lung cancer mortality from exposure to a late-stage carcinogen since they have had time to accumulate more cells in the earlier stages of the cancer process, such cells being particularly susceptible to a late-stage carcinogen such as arsenic. Since a late-stage carcinogen cannot increase the number of cells in the early stages of carcinogenesis, the individual's risk remains constant after cessation of exposure. Following the simple multistage model, we would then explain the synergistic effect of cigarette smoke and arsenic observed by Pershagen as an interaction between the effect on a late-stage of carcinogenesis by arsenic and the effect on an early stage of carcinogenesis by components of cigarette smoke.

The simple multistage model that we have discussed would conclude:

1. That carcinogenic synergism in mixtures is a result of constituents of the mixture acting on separate stages of the multistage process of carcinogenesis.
2. If all constituents of the mixture act on a single stage of the multistage process of carcinogenesis, there will be no synergism. However, an antagonistic effect could result due to the availability of partially transformed cells.

CONCLUSION

Obviously, the simple model cannot explain all of the synergistic or antagonistic carcinogenic effects observed in animal or human studies. As stated earlier, the model does not consider changes in transition rates between stages that may be brought on by age or environmental factors. It is a mathematical model, however, that could certainly explain some of the data. Thus, we feel that implications from the multistage model should be considered in the design of future animal studies or even short-term bioassay studies. In regard to animal bioassays, we would suggest that mixtures be fractionated and administered to the animals, varying the age at which the dose is given and, perhaps, the duration of

the dose. In addition, epidemiologic data should be reported whenever possible to facilitate analysis with regard to the affected carcinogenic stage or stages on which the complex mixture may be acting. The data reported by Doll (1971a) and Brown and Chu (1983) have provided insight with regard to the effects of carcinogens on different stages.

Perhaps one final point should be offered with regard to the understanding of mixtures and components of mixtures by their carcinogenic stage of action. The effects of a late-stage carcinogen would be seen in a relatively short period of time, whereas the effects of an early-stage carcinogen may take many years to be detected. These effects may affect the way we regulate complex mixtures and certainly, we hope, should affect the way in which we study such mixtures.

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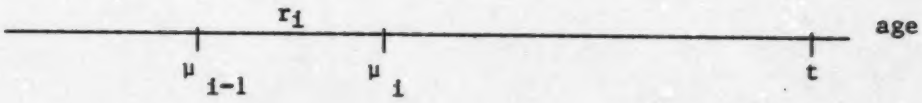
r_i
age
μ_{i-1}
μ_i
t

Figure 1. Schematic view of the transition rate of the i^{th} change in the simple multistage model.

Assumptions: $r_i = a_i + b_i D(u)$, transition rate from $(i-1)^{th}$ stage to i^{th} stage, where a_i is the background rate and b_i is the proportionality constant for the dose.

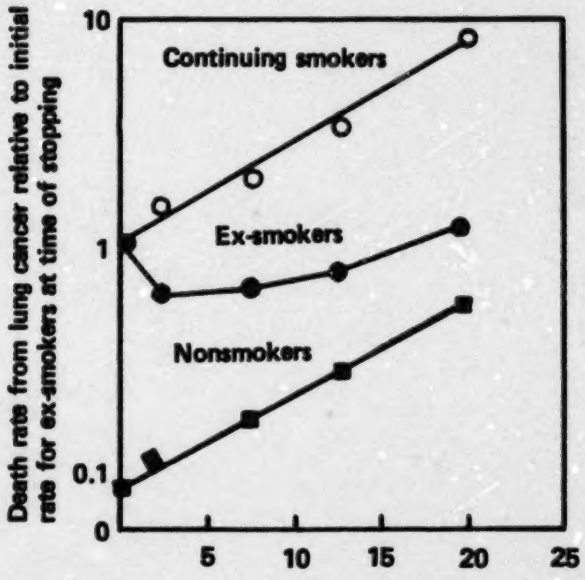
Death rate from lung cancer relative to initial rate for ex-smokers at time of stopping
10
1
0.1
0
Continuing smokers
Ex-smokers
Nonsmokers
5
10
15
20
25

Figure 2. The rate of lung cancer among people who have stopped smoking cigarettes, those who continue to smoke, and those who have never smoked.

Table 1. Examples of Synergism with regard to Carcinogen Response in Human Studies

Agents Involved	Type of Study	Tumor Site	Authors
Smoking and asbestos	Cohort	Lung	Selikoff et al. (1968) Hammond et al. (1979)
Uranium mining and cigarette smoking	Cohort	Lung	Lundin et al. (1969)
Radiation and smoking	Cohort	Lung	Wanebo et al. (1968)
Arsenic and smoking	Cohort	Lung	Pershagen (1982)

Table 2. Examples of Synergism and Antagonism with regard to Carcinogen Response in Animal Studies

Agents Involved	Type of Study	Authors
Synergism		
7,12-DMBA and extracts of unburned cigarette tobacco	Mouse skin painting	Bock et al. (1964)
7,12-DMBA and each of the following: catechol, pyrogallol, decane, indecane, pyrene, benzo[e]pyrene, and fluoranthene	Mouse skin painting	Van Duuren and Goldschmidt (1976)
Automobile exhaust condensate without particulate matter and Benzo[a]pyrene (B[a]P)	Mouse subcutaneous injection	Grimmer (1977)
Antagonism		
Automobile exhaust condensate with particulate matter and B[a]P	Mouse subcutaneous injection	Grimmer (1977)
B[a]P and 10 different non-carcinogens	Mouse subcutaneous injection	Falk et al. (1964)
B[a]P and esculin, quercetin and squalene, and oleic acid (tobacco smoke components)	Mouse skin painting	Van Duuren and Goldschmidt (1976)